PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

Penicillins, Esters, Amides and Salts thereof

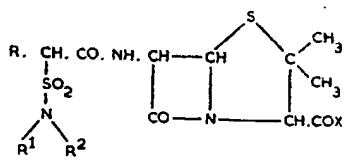
We, BEECHAM GROUP LIMITED, a British Company, of Beecham House, Great West Road, Brentford, Middlesex, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to penicillins, esters, amides and salts thereof and is particularly concerned with α-sulphamoyl-penicillins and their derivatives.

These new penicillins are of value as antibacterial agents, as nutritional supplements in animal feeds, as agents for the treatment of mastitis in cattle and as therapeutic agents in poultry and animals, including man, in the treatment especially of infectious diseases caused by Gram-positive and in some cases Gram-negative bacteria.

Some of the penicillins and penicillin derivatives of the present invention, in addition to their potent antibacterial activity, exhibit resistance to destruction by penicillinase and are thereby effective against resistant strains of bacteria.

Accordingly, the present invention provides penicillins and penicillin derivatives of the general formula:—



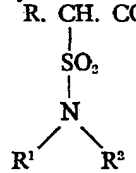
(I)

and non-toxic salts thereof, where R is hydrogen or an alkyl, aryl or heterocyclic group which may be substituted, R^1 and R^2 are the same or different and are each hydrogen or a lower alkyl group and X is an hydroxyl, lower alkoxy, amino or substituted amino group.

The term "lower" as used herein refers to radicals containing from 1 to 5 carbon atoms.

The salts, which term covers both salts of the carboxyl group (I; X=OH) and salts of the acidic sulphonamide group (I; R^1 or R^2=H), include non-toxic metallic salts such as sodium, potassium, calcium and aluminium, ammonium and substituted ammonium salts, e.g. salts of such non-toxic amines as trialkylamines, including triethylamine, procaine, dibenzylamine, N-benzyl-beta-phenethylamine, 1 - ephenamine, N,N' - dibenzylethylenediamine, dehydroabietylamine, N,N' - bisdehydroabietylethylenediamine, and other amines which have been used to form salts with benzylpenicillin.

The penicillins (I; X=OH) may be prepared by treating 6-aminopenicillanic acid or a salt thereof with a reactive derivative of a carboxylic acid of the general formula:—



(II)

where R, R^1 and R^2 have the meanings given above.

Examples of reactive derivatives include the acid halides, azides, anhydrides and mixed anhydrides, and reactive intermediates formed from the acid and a carbodiimide or carbonyldiimidazole.

The penicillin derivatives (I; X=alkoxy

or amino) may be prepared by treating an appropriate ester or amide of 6-aminopenicillanic acid with a reactive derivative of the acid (II). Alternatively, these penicillin derivatives may be prepared from the appropriate penicillin (I; X=OH) by converting the carboxyl group thereof into a reactive derivative and reacting this derivative with ammonia or with the appropriate alcohol or amine. Suitable reactive derivatives of the penicillin include the acid chloride, anhydride, mixed anhydride, or the reactive intermediate formed from the penicillin and a carbodiimide or carbonyldiimidazole.

Example 1

(a) Phenyl-α-sulphamoylacetic acid

Magnesium (3.24 g.) in tetrahydrofuran (6.75 ml.) was treated with ethyl bromide (2 drops). The mixture was stirred and a solution of 2-chloropropane (12.4 ml.) in tetrahydrofuran (19.2 ml.) was added dropwise at such a rate that gentle reflux was maintained. After complete addition a solution of benzyl-sulphonamide (5.13 g. 0.03 mol) in tetrahydrofuran (9 ml.) was added dropwise, with stirring, while maintaining gentle reflux. The mixture was stirred for 3 hours at reflux after addition. The resulting solution was poured onto a mixture of crushed solid carbon dioxide (30 g.) in tetrahydrofuran (28 ml.), stirred, and allowed to attain room temperature during 2 hours.

The solution was acidified with 5N hydrochloric acid (64 ml.), the organic layer separated and the aqueous layer extracted with ether (3×30 ml.). The organic layers were combined, dried over anhydrous magnesium sulphate, filtered and evaporated. The residual oil was mixed with ether and extracted with saturated sodium bicarbonate solution. The alkaline extracts were combined, acidified with 5N hydrochloric acid, saturated with sodium chloride and exhaustively extracted with ether. The ether extracts were combined, dried over anhydrous magnesium sulphate and evaporated to dryness. The solid residue 1.8 g. (27.9%), m.p. 128—147°C after two recrystallisations from acetone/benzene had m.p. 162—164°C (d). (Found: C,44.82; H,4.38; N,6.33; S,14.62. $C_9H_{10}NO_4S$ requires C,44.66; H,4.21; N,6.51; S,14.90).

(b) Sodium (α-sulphamoyl)benzylpenicillin

Phenyl α-sulphamoylacetic acid (0.64 g. 0.003 mol.) was mixed with thionyl chloride (4 ml.) and refluxed for 1 hour. The clear solution was evaporated under reduced pressure, then the residue was mixed with dry benzene (5 ml.) and again evaporated to dryness to remove all excess thionyl chloride.

The residual solid, crude phenyl α-sulphamoyl acetyl chloride, dissolved in dry acetone (15 ml.) was added all at once to a stirred solution of 6-aminopenicillanic acid (0.65 g. 0.003 mol.) in water (15 ml.), N sodium hydroxide (3 ml.), acetone (7.5 ml.), and N sodium bicarbonate (4.5 ml.) at 12°C. The mixture was stirred for 2 hours, filtered through "Celite" (Registered Trade Mark) and extracted with ether (3×15 ml.). The ether extracts were discarded.

The aqueous solution was covered with ether (15 ml.), stirred, and adjusted to pH 2 with N hydrochloric acid. The ether layer was separated and the aqueous solution extracted with ether (2×15 ml.). The combined ether extracts were washed with water (5 ml.) and extracted with N sodium bicarbonate to pH 7. The neutral aqueous extract was evaporated under reduced temperature and pressure and the residue dried over phosphorus pentoxide in a vacuum desiccator to give 1.1 g. (84.3%) of product, estimated by colorimetric assay with hydroxylamine to be 83% pure.

This penicillin inhibited typical Gram-positive bacteria (Staph. Oxford and several strains of Streptococci) at a concentration of 0.25 mcg./ml. and also inhibited various Gram-negative organisms (Proteus mirabilis, Salmonella typhi, Shigella flexneri, and Klebsiella pneumoniae) at concentrations of 5 to 12.5 mcg./ml.

Example 2

(α-Sulphamoyl)benzylpenicillinamide

Sodium (α - sulphamoyl)benzylpenicillin (2.77 g.) was suspended, with stirring, in dry acetone (20 ml.). The mixture was cooled to −5°C, treated with ethyl chloroformate (0.61 ml.) and pyridine (1 drop) and stirred at −5°C for ½ hour. Aqueous ammonia (0.427 ml., d.0.88) in acetone (7 ml.) was added and the mixture was stirred at room temperature for 2 hours. Precipitated sodium chloride was filtered off, and the filtrate evaporated under reduced temperature and pressure. The residue was dissolved in ethyl acetate (30 ml.), washed with water (7 ml.), N sodium bicarbonate (7 ml.), followed by water (7 ml.), and dried over anhydrous magnesium sulphate. The dried solution was evaporated under reduced temperature and pressure and the residual gum triturated with water until solid. The product was filtered off, washed with water and dried over phosphorus pentoxide in a vacuum desiccator to give 2.03 g. of the amide, estimated by colorimetric assay with hydroxylamine to be 91% pure.

This amide was essentially stable towards penicillinase. It inhibited Staph. Oxford at a concentration of 0.1 mcg./ml. and a typical "resistant" (i.e. penicillinase-producing) strain of Staphylococcus at 0.5 mcg./ml. In contrast to the parent penicillin it had very little activity against typical Gram-negative organisms.

EXAMPLE 3

Methyl (α-Sulphamyl)benzylpenicillinate

Sodium (α - sulphamoyl)benzylpenicillin (4.36 g., 0.01 mol.) dissolved in water (25 ml.) was covered with ether (30 ml.) and acidified to pH 2.3 with N-hydrochloric acid (10 ml.). The ether layer was separated and the aqueous layer extracted with ether (2×20 ml.). The ether layers were combined and washed with water (15 ml.).

Ether (50 ml.) was added to a cooled solution of potassium hydroxide (12.5 g.) in water (25 ml.) and the mixture cooled to 5°C. With continued cooling and stirring nitrosomethylurea (5.1 g.) was added portion wise during 1 minute. The yellow ether solution of diazomethane was decanted directly into the penicillin free acid solution, and after 12 minutes excess diazomethane was decomposed with dilute acetic acid. The aqueous layer was separated and discarded. The ether solution was washed with N sodium bicarbonate solution until neutral and finally with water. The solution was dried over anhydrous magnesium sulphate and evaporated under reduced temperature and pressure. The oily residue was dissolved in ethyl acetate (10 ml.) and allowed to crystallise. The solid was filtered off, washed with ethyl acetate and dried to give a colourless crystalline solid 0.48 g. (11.2%) m.p. 144—146°C.

This penicillin ester inhibited Staph. Oxford at a concentration of 2.5 mcg./ml. and several strains of Streptococci at 1.25 mcg./ml., but it had only poor activity against penicillinase-producing "resistant" Staphylococci and Gram-negative bacteria.

EXAMPLE 4

(a) α - (N,N - Dimethylsulphamoyl)phenylacetic acid

Magnesium (2.52 g.) in tetrahydrofuran (5 ml.) was treated with ethyl bromide (2 drops). The mixture was stirred and a solution of 2-chloropropane (9.64 ml.) in tetrahydrofuran (15 ml.) was added at such a rate that gentle reflux was maintained. After complete addition a solution of N,N dimethylbenzylsulphonamide (14 g.) in tetrahydrofuran (60 ml.) was added dropwise, with stirring, while maintaining gentle reflux. The mixture was then stirred at reflux for 3 hours. The hot solution was poured, with stirring, onto a slurry of solid carbon dioxide (30 g.) in tetrahydrofuran (30 ml.) and the mixture allowed to warm to room temperature. The solution was acidified with 5N hydrochloric acid (30 ml.) and the organic layer separated. The aqueous layer was extracted with ether (3×30 ml.) and the organic layers were combined, dried over anhydrous magnesium sulphate, and evaporated. The oily residue was diluted with dry benzene and allowed to crystallise. The solid was filtered off, washed with dry benzene and dried to give a colourless crystalline solid 5.17 g. (30.3%) m.p. 116—118° C (d). Found: C,49.42; H,5.56; N,6.04; S,12.62. $C_{10}H_{13}O_4NS$ requires: C,49.37; H,5.38; N,5.76; S,13.18.

(b) α - (N,N - Dimethylsulphamoyl)benzylpenicillin

α - (N,N - Dimethylsulphamoyl)phenylacetic acid (2.34 g., 0.01 mol.), mixed with thionyl chloride (10 ml.) was heated at 75°C for 30 minutes. Excess thionyl chloride was evaporated under reduced pressure. The residue was dissolved in dry benzene (5 ml.) and again evaporated to remove residual thionyl chloride. The residual crude acid chloride, dissolved in dry acetone (50 ml.), was added all at once to a stirred solution of 6-aminopenicillanic acid (2.16 g., 0.01 mol.) in water (50 ml.), N sodium hydroxide (10 ml.), N sodium bicarbonate (15 ml.) and acetone (25 ml.), cooled to 12°C. The mixture was stirred for 2 hours at room temperature, extracted with ether (3×30 ml.), and the ether extracts discarded. The aqueous solution was covered with ether (30 ml.) and acidified with N-hydrochloric acid to pH 2. The ether layer was separated and the aqueous layer extracted with ether (2×30 ml.). The ether extracts were combined, washed with water (2×10 ml.) and extracted with a sodium bicarbonate to pH 7. The neutral aqueous extract was evaporated under reduced temperature and pressure and dried over phosphorus pentoxide *in vacuo*. The product was obtained as a white solid (4.6 g.) with an estimated purity of 79.3% by colourimetric assay with hydroxylamine.

EXAMPLE 5

(a) Sulphamoylacetic acid

Chlorosulphonylacetic acid (15.5 g.) dissolved in dry ether (200 ml.) was added slowly, with stirring, to liquid ammonia (60 ml.). The mixture was stirred with cooling for 30 minutes then warmed gently to remove excess ammonia. Ether was decanted from the semi-solid mass and the residue dissolved in the minimum of water. The aqueous solution was acidified to pH 1 with 5N hydrochloric acid, evaporated under reduced temperature and pressure, and the residue dried over phosphorus pentoxide *in vacuo*. The resulting solid was extracted with boiling acetone (3×30 ml.), then the extracts were combined and evaporated to dryness. The residue was recrystallised from acetone/benzene to give a colourless crystalline solid 5.55 g. (40.8%) m.p. 121—123°C. Found: C,17.69; H, 3.69; N,9.87; S,22.54. $C_2H_5O_4NS$ requires C,17.27; H,3.62; N,10.06; S,23.05.

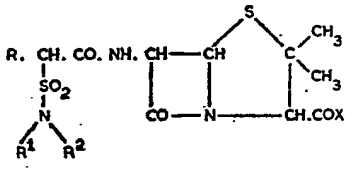
(b) Sodium sulphamoylmethylpenicillin

Sulphamoylacetic acid (1.39 g., 0.01 ml.) in thionyl chloride (10 ml.) was heated at

80°C for 1 hour. Excess thionyl chloride was evaporated under reduced pressure, the residue dissolved in dry benzene (5 ml.) and again evaporated to dryness to remove residual thionyl chloride. The crude sulphamoylacetyl chloride dissolved in dry acetone (50 ml.) was added all at once to a stirred solution of 6-aminopenicillanic acid (2.16 g., 0.01 mol.) in water (50 ml.), N sodium hydroxide (10 ml.), N sodium bicarbonate (15 ml.), and acetone (25 ml.), at 12°C. The mixture was stirred at room temperature for 2 hours, extracted with ether (2×60 ml. and 2×30 ml.), and the ether extracts were discarded. The aqueous solution was covered with ether (40 ml.) and acidified to pH 2 with N hydrochloric acid. The ether layer was separated and the aqueous layer extracted with ether (2×30 ml.). The ether extracts were combined and extracted with N sodium bicarbonate to pH 7. The neutral aqueous extract was evaporated under reduced temperature and pressure and dried over phosphorus pentoxide *in vacuo* giving a cream solid 0.5 g. (13.9%) with an estimated purity of (57%) by colourimetric assay with hydroxylamine.

WHAT WE CLAIM IS:—

1. Penicillins and penicillin derivatives of the general formula:—



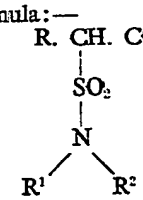
(I)

and non-toxic salts thereof, where R is hydrogen or an alkyl, aryl or heterocyclic group which may be substituted, R^1 and R^2 are the same or different and are each hydrogen or a lower alkyl group and X is an hydroxyl, lower alkoxy, amino or substituted amino group.

2. α - Sulphamoylbenzylpenicillin and non-toxic salts thereof.

3. (α - Sulphamoyl)benzylpenicillinamide.

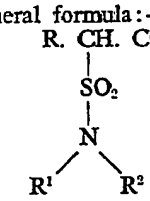
4. A process for the preparation of the penicillins and non-toxic salts thereof claimed in claim 1, wherein 6-aminopenicillanic acid or a salt thereof is treated with a reactive derivative of a carboxylic acid of the general formula:—



(II)

where R, R^1 and R^2 have the meanings given in claim 1.

5. A process for the preparation of the penicillin derivatives claimed in claim 1, wherein an appropriate ester or amide of 6-aminopenicillanic acid is treated with a reactive derivative of a carboxylic acid of the general formula:—



(II)

where R, R^1 and R^2 have the meanings given in claim 1.

6. A process as claimed in claim 4 or claim 5 wherein the reactive derivative of the carboxylic acid is an acid halide, azide, anhydride or mixed anhydride, or a reactive intermediate formed from the acid and a carbodiimide or carbonyldiimidazole.

7. A process for the preparation of the penicillin derivatives claimed in claim 1, wherein a reactive derivative of a penicillin of the general formula (I) is treated with ammonia or with the appropritae alcohol or amine.

8. A process as claimed in claim 7 wherein the reactive derivative of the penicillin is an acid chloride, anhydride or mixed anhydride, or a reactive intermediate formed from the penicillin and a carbodiimide or carbonyldiimidazole.

9. A process for the preparation of penicillins and penicillin derivatives substantially as described with reference to the Examples.

10. Penicillins and penicillin derivatives when prepared by the process claimed in any one of the preceding claims.

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